Indian J. Anim. Nutr. 2025. 42 (1): 119-127
doi: 10.56093/IJAN.v42i1.17

Indian Journal of Animal Nutrition

Performance of Broiler Chicken Supplemented with Zinc oxide Nano Particles

Puja Hazra[1], Pawan Kumar[1], S. Soren[1], I. Samanta[2] and G.P. Mandal[1*],
[1]Department of Animal Nutrition, [2]Department of Veterinary Microbiology
West Bengal University of Animal & Fishery Sciences,
Kolkata, West Bengal 700037.
*Correspondence: gpmandal@gmail.com

ABSTRACT

The study aimed to evaluate the effects of zinc oxide nanoparticles (ZnONPs) supplementation on the growth performance, gut microbiota, and immunity of broiler chickens. Two hundred fifty day-old mixed-sex commercial broiler chicks (Vencobb 400) were randomly divided into five groups with five replicates of 10 chicks each. The dietary groups included: 1) basal diet without Zn supplementation (CON), 2) basal diet with 20 ppm Zn from inorganic ZnO (ZnO20), 3) basal diet with 40 ppm Zn from inorganic ZnO (ZnO40), 4) basal diet with 20 ppm Zn from ZnONPs (ZnONPs 20), and 5) basal diet with 40 ppm Zn from ZnONPs (ZnONPs40).There were no significant differences in body weight, average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), or carcass characteristics among the groups. However, ZnONPs supplementation significantly reduced the count of *Salmonella spp.* ($P<0.001$) and *Clostridium spp.* ($P<0.001$) compared to the control and ZnO groups. Antibody titers against Newcastle disease vaccine were not affected by zinc supplementation. ZnONPs did not impact zinc concentration in tibia and breast tissues but significantly increased zinc concentration in liver tissues compared to ZnO and control groups. Copper concentration in tibia, liver, and breast was not affected by Zn supplementation. In conclusion, ZnONPs supplementation shows promise in broiler production, with beneficial effects on gut microbiota and immunity in broiler chickens.

KEYWORDS: Broiler chickens, Growth , Gut miocrobiota , Immunity, Zinc oxide nano particles

Article received: 17 January 2025; Article accepted: 28 March 2025

INTRODUCTION

Zinc (Zn) is a crucial trace element essential for the overall functioning of all organisms. Zinc is incorporated into chicken diets in inorganic forms, including zinc oxide (ZnO) and zinc sulphate (ZnSO4), as well as organic forms such as zinc acetate, zinc propionate, zinc methionine, and zinc proteinate. However, the bioavailability of zinc from inorganic sources is low, requiring higher levels to meet animal requirements and potentially disrupting mineral balance. The emergence of nanotechnology has introduced zinc oxide nanoparticles (ZnONPs) as a promising alternative to traditional zinc sources in livestock and poultry nutrition. Nanoparticles, with sizes ranging from 1 to 100 nm, possess increased surface area and unique physical, chemical, and biological properties compared to conventional materials. These nano mineral particles offer enhanced bioavailability and absorption due to their larger surface area, allowing for improved interactions with other molecules (Tsai et al., 2016).

Recent studies have highlighted the benefits of ZnONPs in poultry diets, showcasing their superior surface activity, catalytic efficiency, and adsorption qualities compared to conventional sources. Supplementation of ZnONPs at levels of 10 to 100 mg/kg has been shown to enhance broiler growth performance (Zhao et al., 2014; Ahmadi et al., 2017; Yusof et al., 2023). ZnONPs also exhibit antimicrobial properties, with their effectiveness dependent on size and shape, making them versatile for various applications (Siddiqi et al., 2018). Furthermore, ZnONPs offer a potential alternative to antibiotic growth promoters in poultry diets, given their broad-spectrum antimicrobial activity and reduced environmental impact. Research on the effects of zinc nanoparticles on broiler chickens is crucial for optimizing poultry productivity and sustainable agriculture practices. By evaluating the impact of ZnONPs on growth performance, gut microbiota, immunity, and tissue zinc concentrations in broiler chickens, this study aims to provide valuable insights into the role of zinc nanoparticles in poultry nutrition.

MATERIALS AND METHODS

Experimental design and bird husbandry

This study was conducted at the Experimental Poultry Farm in the Department of Animal Nutrition, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, India following CPCSEA Guidelines for Poultry/Bird Facility (2020). Two hundred fifty day-old mixed-sex commercial broiler chicks (Vencobb 400) were randomly divided into five groups with five replicates of 10 chicks each. The dietary groups included: 1) basal diet without Zn supplementation (CON), 2) basal diet with 20 ppm Zn from inorganic ZnO (ZnO20), 3) basal diet with 40 ppm Zn from inorganic ZnO (ZnO40), 4) basal diet with 20 ppm Zn from ZnONPs (ZnONPs 20), and 5) basal diet with 40 ppm Zn from ZnONPs (ZnONPs40).

A basal diet was formulated with maize and soybean meal to meet the nutritional requirements of Vencobb broiler chickens, excluding zinc. The diets were prepared in mash form, with the basal diet lacking supplemental zinc. Zinc oxide (ZnO) from HiMedia Laboratory Pvt. Ltd, Mumbai, India, or zinc oxide nanoparticles (ZnONPs) from the Department of Materials Sciences and Engineering, IIT, Kanpur, India, were added to the basal diet using the top-dressing method and thoroughly mixed. The cryo milling process was used to prepare ZnONPs (Kumar and Biswas, 2015). Chicks were fed a starter diet from 1 to 14 days, a grower diet from 15 to 28 days, and a finisher diet from 29 to 35 days. Feed and water were provided *ad libitum* until 35 days. The ingredient and chemical composition of the basal diet are detailed (Table 1).

Table 1. Ingredient and chemical composition of basal diets

Ingredients (%)	Starter	Grower	Finisher
Maize	62.1	67.9	69.2
Hi Pro Soybean	32.7	27.3	25.5
Soybean oil	1.71	1.54	2.34
Di- Calcium Phosphate	1.36	1.21	0.95
Calcite Powder	0.91	0.88	0.83
Salt	0.27	0.29	0.22
DL-Methionine	0.28	0.26	0.23
Sodium bi carbonate	0.2	0.1	0.2
L-Lysine HCl	0.2	0.24	0.21
L-Threonine	0.11	0.08	0.04
Vitamin premix[1]	0.1	0.1	0.1
Choline Chloride 60%	0.05	0.07	0.07
Coccidiostat	0.03	0.03	0.03
Phytase	0.01	0.01	0.01
Trace mineral premix[2]	0.05	0.05	0.05
Chemical composition (%)			
ME(Kcal/kg)[3]	2925	3025	3100
Crude Protein[4]	21.6	19.3	18.7
Ether Extract[4]	4.57	4.62	5.50
Calcium[4]	0.91	0.85	0.75
Available Phosphorous[3]	0.45	0.42	0.38
Dig. Lysine[3]	1.22	1.12	1.05
Dig. Methionine[3]	0.58	0.54	0.50
Dig. M+C[3]	0.91	0.85	0.80
Dig. Threonine[3]	0.83	0.73	0.66
Zn (mg/kg) [4]	54.4	46.0	44.8
Cu (mg/kg) [4]	6.64	7.58	6.78

[1]Contains (per kilogram): vitamin A, 80,000,000 IU; vitamin D3, 16,000,000 I.U; vitamin E, 64 g; vitamin K, 8 g; vitamin B1, 6.4 g; vitamin B2, 40 g, niacin, 96 g, pantothenic acid, 64 g; vitamin B6, 12.8 g; folic acid 6.4 g; vitamin B12, 0.164 g; and biotin, 0.24g. [2]Contains (per kilogram): 10 g Cu from $CuSO_4$, 80 g Fe from $FeSO_4$, 80 g Mn from $MnSO_4$, 0.70 g I from KI and 0.2 g Se from Sodium Selenite. [3]Calculated value. [4] Analysed value.

The birds were housed in the experimental poultry farm under standard hygienic, management conditions and strict bio-security measures, with each treatment group maintained in their respective pens.

The initial body weight (BW) of all chickens was recorded on the first day of the trial. Weekly weight measurements were taken, with a final measurement on the last day in the morning. Average daily gain (ADG) was calculated for each replicate. Weekly feed intake was determined by subtracting the remaining feed from the total offered per pen. Average daily feed intake (ADFI) was calculated by dividing the total feed consumed per day by the number of chickens in each pen. Feed conversion ratio (FCR) was calculated by considering cumulative feed intake and weight gain for each replicate pen.

One male and one female bird from each replicate, selected based on average body weight, were slaughtered by cervical disarticulation for carcass trait and gut health evaluation. The hot carcass weight was measured without skin and giblets, and various parts were weighed. Dressing percentage and the proportion of different cut parts were measured and expressed as a percentage of slaughter body weight.

Blood samples were collected from one randomly selected bird from each replicate on days 28 and 35 of the trial. A 2 ml blood sample was obtained via vena brachialis puncture under the bird's wing using sterile syringes and needles. The samples were then transferred to centrifuge tubes without anticoagulant and left at room temperature for 1 hour. After centrifugation at 2500 rpm for 5 minutes, clear serum was collected and stored at -20°C for antibody titres analysis. Antibody titres against Newcastle disease virus vaccine at 28 and 35 days were determined using an Antibody ELISA Kit from IDEXX Laboratories, Inc, USA.

The caecal contents of the birds in each treatment group were collected aseptically after slaughter on the 35th day of the experimental trial. After carefully removing the caecal contents, they were stored at 4°C. Bacteriological enumeration was conducted on the same day. One gram of caecal sample was serially diluted tenfold with sterile phosphate-buffered saline (PBS) solution. From the 4- and 5-fold dilutions, 10 µl was plated on EMB agar, *Clostridial* agar, *Lactobacilli* agar, and BGA for E. coli, *Clostridium* spp, *Lactobacilli* spp, and Salmonella spp, respectively (all from HiMedia Laboratory Pvt. Ltd, Mumbai, India). The plates were then incubated aerobically at 37°C for 24 hours, except for the Lactobacillus spp plate, which was incubated for 48 hours. The *Clostridial* agar plates were incubated anaerobically at 37°C. Colonies of each pathogen were counted using a colony counter (hand held digital colony counter LA663, Hi Media Lab Pvt. Ltd., Mumbai, India), and the results were expressed as Log10 colony-forming units (CFU) per gram of sample.

To determine the mineral concentration in tissues, the left drumstick, breast, and liver were collected separately after slaughter and stored at -20°C until analysis. The tibia was extracted and dried in a hot air oven at 100°C until a constant weight was achieved. Subsequently, the tibia, breast, and liver samples were ashed in a muffle furnace for 3 hours at 550°C. The resulting ash samples were dissolved in 0.5 N nitric acid (HNO_3), heated on a hot plate, cooled to room temperature, and filtered using Whatman filter paper No.1. The zinc (Zn) and copper (Cu) content in the digested samples were quantified using an Atomic Absorption Spectrophotometer (PerkinElmer (India) Pvt. Ltd., Kolkata, India) with an air-acetylene flame.

The feed samples were analyzed using the methods outlined by AOAC (1995). Calcium content was determined following the procedure described by Talapatra et al.(1940). Additionally, the AIA levels of the diets were measured using the technique outlined by Furuichi and Takahashi (1981).

The data were analysed by one-way analysis of variance (ANOVA) using SPSS (2017) in a completely randomized design with a model containing treatment taken as the main effect. Single pen was used as an experimental unit. Probability values of $P \leq 0.05$ were declared as significant. When treatment effect was significant, the differences among the treatment means were detected using Duncan's multiple range test.

RESULTS AND DISCUSSION

Growth performance

The BW during the starter, grower, and finisher periods were not significantly affected by zinc supplementation from either zinc oxide (ZnO) or zinc oxide nanoparticles (ZnONPs) ($P>0.05$). Similarly, ADG, ADFI, and FCR were not significantly different between the groups during the starter (1-14 days), grower (15-28 days), finisher (29-35 days), and

overall (1-35 days) periods (Table 2). These results suggest that both nano Zn (ZnONPs) and inorganic Zn (ZnO) supplementation did not impact the growth performance of broiler chickens.

This result align with previous studies by Yusof et al. (2023) and Ramiah et al.(2019). However, in a recent study, Mahmoud et al.(2020) observed higher body weight gain in the 10 mg/kg ZnONPs group compared to the control and other ZnONPs groups. Huang et al.(2007) suggested that optimal live weight gain may be achieved with diet containing 40 mg/kg Zn, as recommended by NRC (1994). In this study, the zinc concentrations in the starter (54.44 mg/kg), grower (46.04 mg/kg) and finisher (44.80 mg/kg) diets were close to the NRC (1994) recommendation.

Table 2. Effects of zinc oxide nanoparticles (ZnONPs) supplementation on growth performance in broiler chickens.

	Treatment[1]					SEM	P-value
	Control	ZnO 20	ZnO 40	ZnONPs 20	ZnONPs 40		
				BW (g)			
Day 14	565.05	552.40	584.10	567.13	573.25	4.201	0.186
Day 28	1529.35	1525.98	1579.18	1534.48	1549.88	11.769	0.646
Day 35	2146.85	2121.25	2126.38	2136.33	2151.93	18.336	0.987
				ADG (g/d)			
Day 1 to 14	37.16	36.19	38.43	37.11	37.83	0.311	0.208
Day 15 to 28	68.88	69.54	71.08	69.10	69.76	0.682	0.893
Day 29 to 35	88.22	85.04	78.17	85.98	86.01	1.701	0.432
Day 1 to 35	60.06	59.30	59.44	59.68	60.24	0.528	0.983
				ADFI (g/d)			
Day 1 to 14	46.53	45.66	47.16	47.13	47.30	0.456	0.812
Day 15 to 28	104.71	106.97	106.71	104.16	106.96	0.905	0.811
Day 29 to 35	153.69	152.12	138.33	146.62	152.53	2.596	0.321
Day 1 to 35	91.23	91.48	89.22	89.84	92.21	0.816	0.809
				FCR			
Day 1 to 14	1.25	1.26	1.23	1.27	1.25	0.009	0.739
Day 15 to 28	1.52	1.54	1.50	1.51	1.54	0.007	0.437
Day 29 to 35	1.75	1.79	1.77	1.71	1.77	0.015	0.541
Day 1 to 35	1.52	1.54	1.50	1.51	1.53	0.006	0.226

[1]Control, basal diet; ZnO 20, basal diet + supplemental Zn at 20 mg/kg feed form ZnO; ZnO 40, basal diet + supplementalZn at 40 mg/kg feed from ZnO; ZnONPs 20, basal diet +supplementalZn at 20 mg/kg feed from ZnONPs; ZnONPs 40, basal diet+ supplementalZn at 40 mg/kg feed from ZnONPs.

Carcass characteristics

There were no significant differences (P>0.05) in the percentage of breast, frame, thigh, drumstick, wing, neck, gizzard, heart, liver, abdominal fat, bursa and spleen relative to slaughter body weight among the groups (Table 3). In contrast, Mahmoud et al.(2020)showed that adding ZnONPs (20 mg/kg) to the basal diet increased eviscerated body weight in broiler chickens. Dukare Sagar et al.(2018) also reported increased immune organs weights (bursa, spleen and thymus) in broiler chickens fed nano Zn particles (40 -80 mg/kg). However, our study found no significant effects of heart and liver relative weight, consistent with previous research (Ahmadi et al., 2017; Mohammadi et al,2015).Eskandani et al.(2021) observed that a combination of 50 mg of nano-ZnO and 2g of *Curcuma longa* /kg improved carcass quality, increased protein and decreased fat in broiler breast and thigh. Siddiqi et al.(2018) suggested that dietary Zn supplementation could be reduced to 20 mg/kg when using ZnONPs without affecting growth performance and carcass traits.

Table 3. Effects of zinc oxide nanoparticles (ZnONPs) supplementation on carcass characteristics as percent (%) slaughter body weight in broiler chickens.

	Treatment[1]					SEM	P-value
	Control	ZnO 20	ZnO 40	ZnONPs 20	ZnONPs 40		
Slaughter BW (g)	2123.50	2130.00	2138.75	2157.50	2132.75	22.258	0.949
Dressing %	66.72	66.95	65.02	66.91	64.10	0.423	0.116
Breast (%)	25.46	25.82	24.57	24.38	23.13	0.386	0.183
Frame (%)	10.77	11.82	11.24	11.99	11.30	0.154	0.091
Thigh (%)	9.04	9.05	8.43	8.48	8.72	0.144	0.522
Drumstick (%)	9.35	9.38	9.53	9.27	9.17	0.098	0.892
Wing (%)	6.39	5.81	5.46	5.76	5.77	0.104	0.120
Neck (%)	2.99	2.85	2.70	2.34	2.58	0.077	0.095
Gizzard (%)	1.98	2.13	2.09	2.19	1.99	0.048	0.663
Liver (%)	1.85	1.85	1.83	1.87	2.04	0.063	0.889
Heart (%)	0.54	0.63	0.52	0.49	0.59	0.023	0.227
Giblets (%)	4.36	4.61	4.44	4.56	4.63	0.109	0.946
Abdominal fat (%)	1.73	1.55	1.75	1.94	1.97	0.075	0.318
Spleen (%)	0.11	0.10	0.11	0.10	0.12	0.005	0.880
Bursa (%)	0.06	0.05	0.09	0.08	0.09	0.009	0.532

[1]Control, basal diet; ZnO 20, basal diet + supplemental Zn at 20 mg/kg feed form ZnO; ZnO 40, basal diet + supplemental Zn at 40 mg/kg feed from ZnO; ZnONPs 20, basal diet +supplemental Zn at 20 mg/kg feed from ZnONPs; ZnONPs 40, basal diet+ supplemental Zn at 40 mg/kg feed from ZnONPs.

Caecal bacterial count

There were no significant differences in the numbers of *Escherichia coli* and *Lactobacillus* spp. observed (P>0.05) (Table 4). Supplementation of ZnONPs at 20 and 40 mg/kg significantly decreased the count of *Salmonella* spp. compared to the control group (P<0.001). The ZnONPs 40 group showed a lower count of *Salmonella* spp. compared to the ZnO20, ZnO40, and ZnONPs20 groups. The counts of *Clostridium* spp. were reduced (P<0.01) with the supplementation of either 20 or 40 mg/kg ZnONPs compared to the control, ZnO20, and ZnO40 groups. Overall, ZnONPs supplementation improved intestinal health by reducing pathogenic bacterial counts in the intestine.

Previous researchers have reported the antimicrobial action of metal oxide nanoparticles; Mahmoud et al., 2020; Yusof et al., 2023; Hatab et al., 2024). However, the exact mechanism by which nanoparticles inhibit bacterial growth is not fully

understood. Elumalai et al.(2015) proposed that bacterial cell death by ZnONPs is caused by the release of ions that combine with thiol groups of proteins on the cell surfaceleading to protein inactivation, decreased membrane permeability and ultimately cellular death. In the present experiment, ZnONPs reduced the caecal population of *Salmonella* spp and *Clostridium* spp. Similarly, Mahmoud et al.(2020)observed that supplementation with 10, 30 and 40 mg/kg ZnONPs decreased the population of total anaerobic bacteria and coliform in the caecum of broiler chickens. Therefore, the antibacterial effect of ZnONPs observed in this experiment is promising and suggests the potential inclusion of ZnONPs in poultry rations as a replacement for antibiotic growth promoters.

Table 4. Effects of zinc oxide nanoparticles (ZnONPs) supplementation on viable bacteria numbers (log10 CFU/ g) in cecal content in broiler chickens.

	Treatment[1]					SEM	P-value
	Control	ZnO 20	ZnO 40	ZnONPs 20	ZnONPs 40		
Escherichia coli	7.55	7.30	7.08	7.33	7.48	0.142	0.883
Salmonella spp	7.65 [a]	7.28 [ab]	7.10 [ab]	6.83[b]	5.85 [c]	0.156	0.000
Lactobacillus spp.	7.53	7.63	7.73	6.90	6.72	0.176	0.261
Clostridium spp.	8.35[a]	8.23[a]	8.55[a]	7.55[b]	7.68[b]	0.107	0.001

[abc]Means bearing different superscripts in the same row differ significantly (P d" 0.05).
[1]Control, basal diet; ZnO 20, basal diet + supplemental Zn at 20 mg/kg feed form ZnO; ZnO 40, basal diet + supplemental Zn at 40 mg/kg feed from ZnO; ZnONPs 20, basal diet +supplemental Zn at 20 mg/kg feed from ZnONPs; ZnONPs 40, basal diet+ supplemental Zn at 40 mg/kg feed from ZnONPs.

Immune response

Zinc supplementation, whether from ZnO or ZnONPs, did not have a significant effect (P>0.05) on antibody titres at 28 and 35 days in broiler chickens (Table 5).

This findings aligns with a previous study by Mahmoud et al.(2020), which also found no impact of ZnONPs supplementation (10, 20 and 30 mg/kg) on Newcastle disease haem-agglutination inhibition titre (ND-HI) in broiler chickens. However, the above report mentioned that supplementation with 40 mg/ kg ZnONPs significantly decreased ND-HI titre in compared to the control group. In contrast, Ahmadi et al. (2017) found that replacing ZnO with ZnONPs in the diet improved antibody titre against the Newcastle disease vaccine at 21, 28, 35 and 42 days of age in broiler chickens.

Table 5. Effects of zinc oxide nano particles (ZnONPs) supplementation on antibody titres (log_{10}) against Newcastle disease vaccine in broiler chickens.

	Treatment[1]					SEM	P-value
	Control	ZnO 20	ZnO 40	ZnONPs 20	ZnONPs 40		
Day 28	2.74	2.64	2.92	2.65	2.72	0.056	0.527
Day 35	2.99	3.04	3.19	3.07	3.14	0.035	0.501

[1]Control, basal diet; ZnO 20, basal diet + supplemental Zn at 20 mg/kg feed form ZnO; ZnO 40, basal diet + supplemental Zn at 40 mg/kg feed from ZnO; ZnONPs 20, basal diet +supplemental Zn at 20 mg/kg feed from ZnONPs; ZnONPs 40, basal diet+ supplemental Zn at 40 mg/kg feed from ZnONPs.

Mineral concentration in tissues

Zinc levels in the tibia and breast did not show significant differences (p>0.05) across treatments (Table 6). However, Zn concentrations in the liver were significantly higher (p<0.001) in both inorganic and nano Zn supplemented groups compared to the control group. Cu levels in the tibia, liver, and breast were not affected by Zn supplementation from either source.

The increased zinc concentration in the liver of the ZnONPs groups is consistent with findings from previous studies. Ramiah et al.(2019) observed elevated zinc levels in the liver of broiler chickens supplemented with ZnONPs. The use of ZnONPs in animal diets can affect zinc deposition due to the improved bioavailability of nano zinc particles compared to inorganic sources (Ibrahim et al., 2017). Akbari Moghaddam Kakhki et al.(2017) reported higher zinc levels in the liver and muscle of Zn-supplemented chickens, while Liu et al.(2009) noted increased zinc concentrations in the liver and breast of broiler chickens regardless of the zinc source. This indicates that bone is the most sensitive tissue to dietary zinc, accumulating more zinc than the liver and muscle, acting as a functional reserve that can be redistributed during deficiency (Janet et al., 2000). In this study, zinc supplementation did not affect copper concentrations in the tibia, liver and breast muscle. Similarly, Mishra et al.(2014)found no differences in copper levels in the bone, liver and muscles after varying levels of zinc supplementation in broiler chickens.

Table 6. Effects of zinc oxide nanoparticles (ZnONPs) supplementation on zinc and copper concentrations (mg/kg) in tibia, liver and breast in broiler chickens.

	Treatment[1]					SEM	P-value
	Control	ZnO 20	ZnO 40	ZnONPs 20	ZnONPs 40		
				Zinc			
Tibia	58.5	74.7	74.2	80.2	84.9	4.087	0.329
Liver	12.7^{c}	24.9^{b}	23.6^{b}	34.2^{a}	32.7^{a}	2.041	0.000
Breast	13.1	11.2	13.3	14.6	16.6	0.626	0.063
				Copper			
Tibia	1.10	1.33	1.12	1.39	1.36	0.047	0.125
Liver	2.22	2.46	1.96	2.40	2.05	0.135	0.768
Breast	1.05	0.69	0.89	1.12	0.72	0.069	0.146

[1]Control, basal diet; ZnO 20, basal diet + supplemental Zn at 20 mg/kg feed form ZnO; ZnO 40, basal diet + supplemental Zn at 40 mg/kg feed from ZnO; ZnONPs 20, basal diet +supplemental Zn at 20 mg/kg feed from ZnONPs; ZnONPs 40, basal diet+ supplemental Zn at 40 mg/kg feed from ZnONPs.

CONCLUSION

In conclusion, the findings of the study suggest that the supplementation of zinc oxide nanoparticles (ZnONPs) resulted in a notable reduction of pathogenic bacteria residing in the intestinal tract while simultaneously enhancing zinc concentrations within the liver. Despite these positive outcomes, it is important to note that ZnONPs did not yield significant improvements in either the growth performance metrics or the overall immune response of broiler chickens, indicating that while there are benefits related to microbial control and trace mineral status, other performance-related parameters remained unaffected by this supplementation strategy.

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